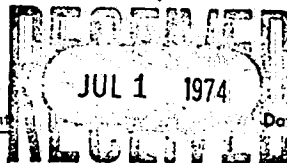


# 833A-

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 59TH STREET  
NEW YORK, N. Y. 10022  
(212) 421-8965



Application for Research Grant  
(Use extra pages as needed)

Date: 6/25/74

1. Principal Investigator (give title and degrees):

A. Stanley Weltman, Ph.D., Associate Professor in Pharmacology and Research

2. Institution & address:

Laboratories for Therapeutic Research  
Brooklyn College of Pharmacy  
Long Island University  
600 Lafayette Avenue, Brooklyn, N. Y. 11216

3. Department(s) where research will be done or collaboration provided:

- a) Laboratories for Therapeutic Research
- b) Institute of Pathology, Downstate Medical Center, S. U. N. Y.  
Brooklyn, N. Y.

4. Short title of study:

Effects of Nicotine and Cholesterol in Spontaneously Hypertensive and Normotensive Rats

5. Proposed starting date: January 1, 1975

6. Estimated time to complete: Two Years

7. Brief description of specific research aims:

As subsequent discussion will illustrate, considerable controversies exist concerning possible deleterious effects of the habit of smoking (nicotine) and/or high cholesterol diets on hypertension, cardiovascular, arteriosclerotic and atherosclerotic pathologies. One can question the progressive effects of these agents not only in normal individuals but those subject to essential hypertension. The Japanese strain of spontaneously hypertensive rats (SHR) has been reported to represent an excellent animal model of essential hypertension. The present investigation therefore proposes to compare and relate interactions of intermediate and prolonged periods of either single and/or combined administration of nicotine alkaloid and high cholesterol diets with systolic blood pressure, plasma lipid profile and the possible etiological development of hypertension, arteriosclerosis and atherosclerosis. Animals to be utilized will be the spontaneously hypertensive rats as well as its comparable genetic normotensive strain. Among the many questions to be resolved are the possible synergistic and antagonistic actions etc., of either the single and/or combined treatments on the blood lipid profile, blood pressure and vascular pathologies, etc. in the SH and normotensive strains. Doses employed will be 2.28 mg/kg/day of nicotine alkaloid administered in drinking water (equivalent to 2 packs of cigarettes per day) and a Purina Lab Chow Meal mix containing an additional 5% cholesterol supplement.

In previous studies, our laboratory has demonstrated that 2.28 mg/kg/ of nicotine alkaloid administered to SHR for 6 and 29 weeks caused decreases in body weights, consistent trends of lower systolic blood pressure levels by the 4th week and significant decreases in plasma cholesterol levels of rats sacrificed after 6 and 29 weeks

(SEE ATTACHED PAGES 6-13)

1003544380

8. Brief statement of working hypothesis:

Our present proposal aims to determine if nicotine decreases cholesterol, affects lipid metabolism and diminishes hepatic cholesterol synthesis in the spontaneously hypertensive rats. Concomittant studies would explore the effect in genetically related normotensive male rats. In addition, the study proposes to clarify interactions and effects of nicotine and cholesterol intake in the progressive development of hypertension in initially hypertensive and normotensive animals.

Considerable controversy exists concerning the effects of smoking and/or nicotine on cholesterol levels as well as the relationships of high cholesterol intake with the development of hypertension, vascular pathology, atherosclerosis etc. Several investigators have reported higher cholesterol levels in smokers. Findings by Svacha et al (58) of effects of egg intake and tobacco smoking on serum cholesterol levels appeared to indicate that serum cholesterol responses to egg intake was amplified by smoking. While numerous reports have indicated associations of cholesterol with hypertension, vascular pathology, atherosclerosis etc., Nagaoka et al (114) claimed that feeding of high cholesterol diets (2%) plus 0.15% propylthiouracil or a diet of 2% cholesterol to spontaneously hypertensive rats indicated that cholesterol was not involved in the progression of hypertension nor in the occurrence of thrombus formations.

(see attached page 14)

9. Details of experimental design and procedures (append extra pages as necessary)

Mature male spontaneously hypertensive rats (SHR-Series I) and genetically related normotensive Wistar rats (WKY/N-Series II) approximately 10 weeks of age will be selected for the 6 month and 1½ year studies. Within each series, the hypertensive and normotensive animals will be matched by body weights and divided into 4 groups: Group A-nicotine-treated (2.28 mg/kg/day); Group B-cholesterol (5%); Group C-nicotine and cholesterol regimens; Group D-untreated controls. The rats in each group will be housed 4 per cage in duprolene cages (22" x 15" x 7") provided with stainless steel lids with feed and water compartments and a removable stainless steel grid floor. Trays placed beneath the hanging cages and provided with Sani-chips will permit sanitary maintenance of the cages. During an initial two week period, animals will be permitted to eat and drink water ad libitum. Water consumption will be measured daily via the use of graduated water bottles. Measured quantities of food will be provided to all groups consisting of Purina Laboratory Chow Meal and the total food consumption and water consumption will be evaluated during the respective weekly periods. Analysis of food consumption and body weight gain relationship obtained via weekly body weight measurements will permit evaluation of food utilization ratios (body weight gain (gm)/food consumed (gm) in the respective treated and control spontaneously hypertensive groups.

After the first week of acclimatization, base line studies of systolic blood pressures will be obtained. The indirect measurement of systolic pressure will be obtained via the tail-cuff procedure and by means of a pulse transducer (146). Readings will be obtained with a Narco-Biosystems Physiograph (Desk Model-DMP-4B) from warmed, unanesthetized rats. Prior to placing the individual rats in a Narco-Biosystems Inc. plastic restraining cage warming board unit (37°C), all animals will be individually prewarmed in an incubator maintained at 37°C for 15 minutes. As employed at our laboratory blood pressure levels will be based on averages derived from 10 readings.

At the completion of the 2 week period and at approximately 12 weeks of age, nicotine and cholesterol treatment will be initiated for the respective groups. On the basis of instructions for chronic administration of nicotine used by the Dr. Wenzel laboratory (private communication), the daily dose of nicotine will be provided in a volume of water that the group of animals will consume in approximately 18 hours, taking into account the volume of water lost by dripping. Our laboratory provides the proper amount of drinking water with nicotine to the animals at 3:00 pm and at 9:00 am the following morning water bottles are checked, water consumption estimated and nicotine-containing bottles, replaced with measured quantities of fresh tap water for the remaining 6 hours. During the 18 hour period on the basis of water consumption and body weight measurements nicotine alkaloid drinking solutions will be replaced to provide a daily oral intake of 2.28 mg/kg body weight

(see attached pages 15-24)

1003544381

## 10. Space and facilities available (when elsewhere than item 2 indicates, state location):

a) The space and facilities available at the Laboratories for Therapeutic Research, Brooklyn College of Pharmacy are as follows:

The Laboratories were designed for the purpose of conducting animal investigations in physiology, pharmacology, endocrinology, biochemistry, and experimental therapeutics. It is a Laboratory which is equipped for work in all of these fields and possesses histological, microscopic, biochemical and animal-surgical equipment necessary for the conduct of detailed investigations.

The animal rooms are air-conditioned, the animals are housed in metal cages and an automatic cage-washing machine is available. The permanent equipment in addition to the cages includes: (1) Leitz Ortholux Binocular Microscope, (1) Sartorius Selecta Precision balance, (1) F.P.E. Precision balance, (2) ovens, (2) Incubators, (2) Refrigerators, (1) Freezer, (1) Turner Fluorometer, Model 110, (1) Coleman Spectrophotometer Model 6, (1) Spectronic 20 (Bausch & Lomb), (1) Servall Centrifuge, (1) Adams Dynac Centrifuge, (1) Torbal Torsion Balance, (1) Bausch & Lomb freezing and (1) Spencer rotary paraffin microtome, a Technicon for processing histological specimens, (1) Beckman pH Meter, (2) A.H. Thomas shakers, (1) Demineralizer Unit (Barnstead), (1) Corning AG-1 Glass Distilling Apparatus, (1) Hot plate, (1) Stir-Jack, (1) Elconap Constant Temperature Water Bath, (1) Friden Calculator, (1) Friden 130 Electronic Calculator, (1) Marchant Cogito 566 PR Calculator, (1) General Radio Oscillator, Type 1210 C and amplifier, (1) Audiogenic Stress Belling Chamber, (1) Stainless Steel Pipette Washer and a miscellany of glassware and accessory equipment. (1) Narco-Biosystems, Desk Model DMP-4B, Physiograph and accessory equipment for systolic blood pressure measurements. In addition to a fluorometer, spectrophotometer and a Micrometric Syringe Microburet (Model SB 22) equipment for the various biochemical (see attached page 25)

## 11. Additional facilities required:

## 12. Biographical sketches of investigator(s) and other professional personnel (append):

A.S. Weltman (pages 26-30), N.Y. Mary (pages 31,32), V.M. Yermakov (pages 33-35), S. Schwan (pages 36,37)

## 13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

(see page 38)

1003544382

14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s)  
even if no salary requested)

	% time	Amount
A. Stanley Weltman, Ph.D.	25	N.C.
Nouri Y. Mary, Ph.D.	15	N.C.
*Valentin M. Yermakov, M.D.	10	N.C.
*Stefan Schwan, M.D.	15	N.C.

\* Pending agreement to pursue pathological study, as a supplemental grant after completion and evaluation of biochemical and blood pressure data (see section 9 page 16)

Technical

	% time	Amount
Vijay Pandhi, M.S.	100	13,000
Leroy Johnson, B.S.	75	7,500
Ratilal Vaidya, B.S.	50	5,000
Caretaker	30	2,210
(Pathology Technicians Pending Supplemental needs)	---	---

Sub-Total for A \$ 27,710

B. Consumable supplies (by major categories)

Feed and Bedding	850
SHR and WKY/N rats (Normotensive)	1,040
Glassware, Chemicals, Lipoprotein assays etc. Physiograph and Densitometer Recording Papers, etc.	1,675

Sub-Total for B \$ 3,565

C. Other expenses (itemize)

Sub-Total for C

Running Total of A + B + C

D. Permanent equipment (itemize)

Animal Rack and Cages	1,000
-----------------------	-------

Sub-Total for D \$ 1,000

E. Indirect costs (15% of A+B+C)

E 4,842

Total request \$37,117

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	\$27,710	\$2,800			\$4,577	\$35,087
Year 3						

1003544383

16. Other sources of financial support:

List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Laboratories for Therapeutic Research Research Institute of The Brooklyn College of Pharmacy, is a non-profit basic research institution at Brooklyn College of Pharmacy. Costs of Plant Operation are jointly shared by the College and the Laboratories. Expenses for the Laboratories Operations are from private contributions.			

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name A. Stanley Weltman, Ph.D.

Signature A. Stanley Weltman Date 6/25/74

Telephone 212 MA 2-1790  
Area Code Number Extension

Responsible officer of institution

Typed Name Seymour Schertz

Title Comptroller

Signature Seymour Schertz Date 6/25/74

Telephone 212 MA 2-4040  
Area Code Number Extension

Checks payable to

Brooklyn College of Pharmacy

Mailing address for checks

600 Lafayette Ave., Brooklyn, N.Y. 11216

1003544384

Item #7. Brief description of specific research aims (continued):

of treatment. Significant decreases in relative liver weights at 29 weeks in conjunction with decreased plasma cholesterol levels suggested the possibility of decreased hepatic cholesterol synthesis in the SHR.

Specifically effects of nicotine and/or cholesterol regimens will be investigated in spontaneously hypertensive and normotensive strains for a 1½ year period (Group I-nicotine; Group II-cholesterol; Group III-nicotine-cholesterol; Group IV-control). Aliquot populations will be sacrificed after 6 months of treatment. The present proposal has the following objectives:

1. Observation of weekly body weights and food consumption data would furnish information on effects of the treatments on body growth and development as well as influences on food metabolism and food utilization processes.
2. Similar measurements of water consumption (daily) and for the total week would be made to ensure intake of the required dose of nicotine/day as well as the comparative effects of the various regimens on water consumption and metabolism in the respective strains.
3. Comparison of base-line systolic blood pressure levels obtained prior to initiation of the treatment and at spaced intervals during the long range studies would determine time-sequential and progressive effects of nicotine and/or cholesterol on the systolic blood pressure levels of the respective groups. In addition effects of age on systolic blood pressure levels can likewise be ascertained.
4. Periodic sampling of blood specimens from the orbital sinus can be obtained for lipoprotein assays (electrophoresis). Time-sequential evaluations can be derived of not only the lipoproteins but also plasma cholesterol, triglyceride, phospholipid and FFA levels indicating the effects of the agents on the cholesterol and lipid profile as well as lipid metabolism. Relationships between cholesterol and lipid levels to the etiology of hypertension as well as peripheral and cardiovascular pathologies, etc. can be determined and evaluated.
5. Similar periodic sampling of orbital sinus specimens can be taken for plasma glucose and total plasma protein assays during the long range period.
6. To ascertain nicotine and nicotine metabolite levels (i.e., cotinine, etc.) as a function of the oral intake of 2.28 mg/kg/day of nicotine, assays will be undertaken using GLC (gas liquid chromatography) procedures to determine the nicotine blood levels, in the treated groups. At autopsy, liver specimens will be extracted for nicotine and metabolite assays.
7. Autopsy of aliquot populations at 6 months and 1½ years after initiation of treatment will demonstrate effects on endocrine and associated as well as vital organs. Thus, the adrenals, thymus, spleen, testes, seminal vesicles, liver, kidneys and hearts will be removed and weighed. The effects of the substances on such organs as the adrenals and gonads and their respective target organs will be evaluated. Similarly, heparinized blood samples obtained after decapitation can be assayed for the lipoprotein, cholesterol, triglyceride, phospholipid, FFA, glucose and total protein.
8. At autopsy, gross examination will be made of the brain, lungs, heart, kidneys, blood vessels etc for pathological observation. In addition, brain, heart, lung, kidneys, aorta, pulmonary artery, renal blood vessels, tissues and organs will be fixed in 10% formalin for appropriate histological evaluation of peripheral, cardiovascular and cerebrovascular arteriosclerotic and atherosclerotic abnormalities due to the respective treatments.
9. Thus, statistical analyses, comparisons and evaluation of blood pressure levels, plasma lipid profile with possible pathological changes after intermediate and chronic treatment will aid in clarifying the controversial aspects and differential hazards which either the single or combined agents may pose to the hypertensive and normotensive strains and possibly to man. Although it is dangerous to extrapolate animal findings to man, the hypertensive rats represent an animal model somewhat comparable to essential hypertension. To date, review of the literature has revealed no evidence wherein nicotine and/or cholesterol effects were simultaneously studied in spontaneously hypertensive rats.

1003544385

Item 7. continued:

Introduction and Background

In the past, the effects of smoking tobacco and its active constituent nicotine have been controversial. Smoking has been accused of being an etiological factor leading to hypertension, coronary heart disease, arteriosclerosis, atherosclerosis, etc. (1-15). It has been claimed that in heavy smokers there is increased mortality due to hypertension and coronary heart disease (6,12,15).

Although the etiology of hypertension, arteriosclerosis and atherosclerosis is complex and obscure and subject to much controversy, emphasis has been placed on the possible causative role of high cholesterol and fat diets in the development of these disease states. Correlations have been reported between abnormally elevated serum lipid levels and the incidence of ischemic cardiovascular disease. The striking association between serum cholesterol levels and atherosclerotic cardiovascular disease in the genetic disorder familial hypercholesterolemia has been well documented (16,17). Some types of hyperlipidemia are known to predispose subjects to coronary heart disease (18,19), peripheral vascular disease (20,21) and a possible relationship to cerebrovascular disease (21,22).

Since Fredrickson and Lees in 1965 (23) and in subsequent reports (24) proposed a system for phenotyping hyperlipoproteinemias, the concept of coronary disease detection and prevention using lipoprotein assays as a clinical tool has become exceedingly prevalent. Findings have suggested that if identified at an early stage coronary-prone individuals may be treated effectively to prevent premature disability and death (25,26). As a result of Fredrickson and Lees studies (23,24), the aberrant states of hyperlipoproteinemias have been classified into 5 types dependent upon variations in the proportions of chylomicrons, alpha lipoprotein, beta and prebeta lipoprotein fractions. It has been claimed that the beta fraction carries the major portion of cholesterol while the prebeta carries the major share of the endogenous triglycerides.

Previously, Wenzel et al (27) reported that 2.28 mg/kg of nicotine alkaloid administered orally to normal female rats and physiologically equivalent to the intake of "two packs of cigarettes per day" induced a biphasic effect on systolic blood pressure by first elevating it for 15 weeks followed by subsequent significant decreases in blood pressure upon continued oral administration. With larger oral doses (equivalent to 3 and 4 packs of cigarettes per day) only depressor effects on systolic blood pressure levels of anesthetized female rats were observed (28). Administration of either "low" or "high" doses of nicotine lowered the systolic pressure of renal hypertensive rats to below control levels (28).

Studies by our laboratory after oral administration of 2.28 mg/kg of nicotine alkaloid to spontaneously hypertensive male rats for six (29) and 29 (30) week periods indicated among other observations reductions in body weights, significant decreases in plasma cholesterol titers but no significant alterations in FFA and suggestive trends of reductions in systolic blood pressure levels. Significant decreases in the relative liver weights in conjunction with the decreases in plasma cholesterol suggested the possibility of decreased hepatic cholesterol synthesis in the 29 week study. Thus, as a consequence of the diverse reports of higher cholesterol levels in smokers (1,2), this aspect as well as detailed assays of the plasma lipid profile warranted further investigation.

In view of reports that smoking caused a larger rise in blood pressure levels of hypertensive subjects than in normal subjects (3) and administration of "low" or "high" doses of nicotine to renal hypertensive rats lowered blood pressure to below control levels (28), our initial quest had been to determine possible hypertensive and/or hypotensive effects of nicotine in the spontaneously hypertensive rats. Indications of reductions in systolic blood pressure levels appeared to suggest that the spontaneously hypertensive rats like like renal-hypertensive rats (28) may possibly be more susceptible to sympathetic ganglionic blockade by nicotine.

1003544386

Investigations by our laboratory of the acute effects (29,31) of subcutaneous administration of nicotine alkaloid (0.5 or 1.0 mg/kg) to spontaneously hypertensive male rats sacrificed at 30 and 60 minute periods indicated that at 30 minutes, nicotine stress via increased adrenal catecholamine release induced mobilization of FFA in addition to elevations in plasma corticosterone. Nicotine-induced increases in cholesterol and hyperglycemia were observed at 60 minutes at which time plasma and adrenal corticosterone levels were decreased. The degree of adrenal depletion of corticosterone produced by acute nicotine stress in the spontaneously hypertensive rats suggested a lower rate of adrenal corticosteroidogenesis.

After 6 and 29 weeks of oral administration of nicotine alkaloid (2.28 mg/kg/day) to the SHR, no significant alterations were noted in either the plasma corticosterone, adrenal corticosterone or adrenal catecholamine concentration. Relative adrenal weights were significantly heavier in the nicotine-treated rats at 29 weeks.

#### CTR833-R1

In our previous progress report/number 3 (June 1973) where nicotine alkaloid was administered orally to normotensive Wistar rats (CFW, Carworth, Inc.) not genetically related to the Japanese spontaneously hypertensive strain, similar significant decreases were noted in the body weights and significant increases in the relative adrenal weights at 29 weeks. Although relative liver weights and plasma cholesterol levels were decreased, these changes were not significant. Trends of slightly lower systolic blood pressure levels were also observed in the Carworth nicotine-treated normotensive rats after the 11th week, but the changes were not significant. At 29 weeks, significant decreases were observed in the plasma glucose levels of the nicotine-treated normotensive rats. Smaller decreases in the glucose levels of the nicotine-treated hypertensive rats were not significant. One may question the contributing effects of the differences in the genetic origin of the Japanese and Carworth strains on the response patterns of the hypertensive and normotensive rats to nicotine. The present proposal involving investigations of the SHR and WKY/N strains will minimize genetic diversity since the SHR and the normotensive WKY/N stocks have the same genetic origin.

#### involved

Additional aspects of the various nicotine investigations/joint collaborative efforts with Drs. Valentin Yermakov and Stefan Schwan, staff members of the Dept. of Pathology at Downstate Medical Center of New York. The objectives were to determine possible increases in peripheral, cardiovascular and cerebrovascular pathology due to nicotine administration in the spontaneously hypertensive rats. To date, continuing histological studies have revealed no evidence of an increase in peripheral, cardiovascular and cerebrovascular pathology which could be attributed to nicotine in the spontaneously hypertensive rats (administration of nicotine for 29 weeks; other studies under examination).

#### Smoking and Body Weights

In terms of smoking effects on body weights, Damon (2) reported lean men smoked significantly more than fat subjects. Karvonen et al (1) reported smokers of rural regions were slightly thinner whereas urban smokers showed no difference in body weights between smokers and nonsmokers. In the Thomas (6) investigations which represented an urban group of medical students smokers have a larger proportion of heavy individuals. In contrast Brozek and Keys (32) reported that men who stopped smoking tended to become fatter. Recent studies (33) have similarly indicated that smokers have lower body weights than nonsmokers and that ex-cigarette smokers gained more weight and were heavier than smokers and nonsmokers.

#### Smoking and/or Nicotine Blood-pressure Studies

It is quite evident from smoking studies in man (7,34) and from animal investigations (35,36) that acute tobacco smoke and/or nicotine can produce transient increases in blood pressure, etc. Whether the habit of smoking tobacco is related to the development of hypertension, coronary heart disease and arteriosclerosis has long been the

1003544387

subject for discussion (2,6,11,12). Yet, in epidemiological studies to correlate cardiovascular changes with smoking, Hadley (14) reported that the average blood pressure of smokers was somewhat less than non-smokers. Hammond and Horn (15) and Damon (2) have also been unable to establish a relation between cigarette smoking and hypertension. Blackburn et al (4) reported distinct tendencies to lower systolic and diastolic blood pressures in chronic smokers but found higher basal and resting pulse rates in smokers. It has also been reported that after smoking 2 cigarettes, habitual smokers show rather prolonged pressure responses and longer sustained increase in heart rates than do non-smokers (10). Thomas (6) further compared characteristics of smokers and non-smokers with family histories and claimed that student smokers presented family histories of parental hypertension more often and had higher mean recumbent values for heart rate and pulse pressure, but noted that student non-smokers had higher mean recumbent values for diastolic pressure. Smoking has also been reported to cause a larger rise in the blood pressure levels of hypertensive subjects (3) than in normal subjects. Recent findings reported by Seltzer in 1974 (33) claimed that cigarette smoking tended to have an inhibitory effect on blood pressure levels. Cigarette smokers presented lower, over-all mean systolic and diastolic blood pressure levels than non-smokers or quitters.

Chronic studies with animals involving effects of nicotine on blood pressure have also been inconsistent. In part, these can possibly be related to dosage, mode of administration, species, etc. Haag et al (36) exposed rats to chronic cigarette smoke for periods up to 2 years and reported lower blood pressure levels that were not statistically significant and no evidence of chronic hypertension. When the blood pressures were determined prior to and after exposure to cigarette smoke, significant increases were noted in the systolic blood pressure which persisted for 45 minutes. In contrast, rabbits given nicotine alkaloid in drinking water, revealed significant and cumulative increases in systolic blood pressure during a 24 week period (37). Similarly, Bhagat (38) administered nicotine subcutaneously to rats daily for 6 weeks and Westfall (39) for 8 weeks; both demonstrated gradual and significant increases in systolic blood pressure as administration continued. Paradoxically, Wenzel (27) also administered nicotine in drinking water to rats and reported significant, cumulative elevations in tail systolic blood pressure up to and through the 15th to 20th weeks, followed by a gradual decline to normal levels at week 30 and significantly lower blood pressure readings at week 55. A similar biphasic effect on blood pressure by nicotine had previously been reported by Kin (40) wherein initial elevations of blood pressure of rabbits were followed by a secondary lowering to below control levels as the treatment with the drug was prolonged. These paradoxical observations of increases in blood pressure of animals followed by decreased levels in the same animals as treatment continued along with similar reports of somewhat lower blood pressure levels in chronic smokers pose interesting questions. Conceivably, these decreases may result from chronic sympathetic blockade or depletion of vascular tissue norepinephrine by nicotine. Similarly, the questions of the development of tolerance and/or facilitation must also be considered. In studies of the reaction of chronic smokers, Roth and Schick (3) reported that habitual smokers did not show tolerance to the immediate effects of smoking as indicated by blood pressure, pulse rate and skin temperature measurements which involved 66 standard smoking tests. In contrast, Thienes (41) reported that rats subjected to pivoting response tests showed the development of tolerance within 2 weeks, no tolerance to convulsive and fatal doses of nicotine and some indications of tolerance development by the adrenal medulla to the stimulating effects of nicotine.

In doses absorbed by cigarette smokers during and shortly after smoking, nicotine the active constituent, has been found to increase heart rate, raise arterial pressure dilate arterial blood vessels of muscles while contracting those of the skin, to increase cardiac output (10) and to reduce the skin temperature of the extremities, etc.

1003544388

(34). Thus nicotine produces a complex array of cardiovascular responses and hemodynamic effects in which the precise mechanisms cannot be readily defined (42). The low dose effects of nicotine have been ascribed to the release of catecholamines from the adrenergic nerve endings of the adrenal medulla (42,43), with stimulation of the sympathetic ganglia, etc. (42). In large doses, nicotine blocks ganglionic transmission and can paralyze ganglion cells. By virtue of its ability to stimulate the parasympathetic nervous system, nicotine can elicit responses opposite to sympathetic stimulation (44,45). The parasympathetic ganglia require higher concentrations than the sympathetic ganglia for direct stimulation (44). Similarly, other diverse and opposing mechanisms can occur, in that while stimulation of the aortic and carotid chemoreceptors causes increases in arterial pressure, heart rate, respiration rates, etc., activation of the pulmonary and coronary artery receptors produce bradycardia, hypotension and apnea (44,45). It is important to note that nicotine action varies with species, as in the cat. Vasoconstriction by nicotine has also been induced by stimulation of the posterior pituitary to secrete antidiuretic hormone and by direct action of nicotine in the blood vessel walls of the arteries (3).

#### Smoking and Cholesterol, etc.

Again conflicting reports have been cited regarding the relationship of chronic smoking habits with blood cholesterol levels. Whereas, Blackburn et al (4) did not observe any significant differences between cholesterol levels of smokers vs non-smokers, others have reported positive correlations and significantly higher blood cholesterol levels in smokers (1,2,6,46). Acute effects in smokers after 2 cigarettes were found to show significant increases in free fatty acids and blood glucose levels but no significant alterations in the blood cholesterol and triglyceride levels (47).

In rabbits, elevated plasma cholesterol levels have been obtained after continued oral administration of nicotine and similar effects after nicotine injections (48,49). Injection of nicotine in dogs for 6 weeks was found to significantly elevate serum cholesterol, although no significant change was observed in serum triglycerides (9). It was suggested that the cholesterol increases were secondary to nicotine induced release and rise of free fatty acids which stimulated increased cholesterol synthesis by the liver. Acute responses of dogs to inhalation effects of cigarette, pipe, cigar smoke, etc. have demonstrated mobilization of serum FFA as well as increased triglyceride levels (50).

#### Smoking and Adrenal Activity

The important role of the adrenal gland and the sympathetic nervous system in control of free fatty acid liberation has been well recognized (51,52). Smoking in man and administration of nicotine to dogs have likewise demonstrated rapid mobilization of free fatty acids from the fat depots due to stimulated adrenal medullary activity and increased catecholamine secretion (53). Tobacco smoke or nicotine administered to cats have similarly demonstrated stimulated release of catecholamines from the adrenal medulla (35).

Adrenocortical stimulation in man by tobacco smoke and also in dogs and rats by acute nicotine administration have likewise been reported (54). It was suggested that the increases in corticosteroid levels resulted secondarily from nicotine-induced catecholamine activity enhancing corticotrophin release. Regarding thyroid function, Blackburn et al (4) indicated no significant difference in blood PBI values of smoker vs nonsmoker subjects.

#### Nicotine, Cholesterol and Atherosclerosis

In studies of atherogenic effects of cholesterol diets, increases in atherosclerosis have been reported in rabbits treated with nicotine (49,55). Stefanovich et al (56) further reported that 2.28 mg/kg of nicotine administered orally to rabbits fed an

1003544389

atherogenic diet increased the degree of aortic atherosclerotic lesions as well as serum cholesterol levels in rabbits at 6 weeks. Wenzel et al (57) however, in rabbits administered nicotine orally at dose levels of 1.14 mg/kg observed no histological and pathological differences between groups given nicotine and an atherogenic diet or the diet alone. This may be a reflection, however, of the lower dose of nicotine which was equivalent to 1 pack of cigarettes per day. It is of interest that recent studies comparing blood cholesterol levels in smokers, nonsmokers and subjects eating 0, 1, and 3 eggs/day revealed that the serum cholesterol response to egg intake was amplified by smoking (58).

#### Chronic Effects of Nicotine

In chronic studies with animals, some investigators have reported harmful effects of chronic nicotine poisoning such as retardation of growth (59-62), adrenal enlargement or injury (59,63,65), interference with reproduction (59,66,67) and atrophy of the gonads (59,63,68). Many of these findings were based on studies of small animal population. Others, exposing rats to tobacco smoke (36) reported no significant differences in blood pressure, longevity, reproduction and pathology.

#### Cholesterol diets and atherosclerosis

Cholesterol diets have produced atherosclerosis in rabbits (69,70) and have been reported as early as 1934 (69). Dauber and Katz (71,72) were the first to show that atherosclerosis could be consistently produced in the chicken by feeding diets high in cholesterol. A correlation was further demonstrated between the degree of atheromatosis development in the chicken and the amount of cholesterol added to the diet (73). Cockrels fed an atherogenic diet demonstrated aortic atherosclerosis as well as elevated plasma cholesterol levels at 27 weeks (74). In rats, the production of cardiac thrombi and infarcts have been induced by the ingestion of thrombogenic diets (high fat-cholesterol diets containing in addition propylthiouracil) (75,77). Other studies have indicated that the degree of spontaneous arteriosclerosis produced by repeated breeding of a strain of male and female rats (78) was augmented by a high fat-cholesterol diet and/or ACTH administration (79). Investigations with cholesterol-fed rats have likewise demonstrated hyperlipoproteinemia with altered distribution in the subunit composition of the serum lipoprotein fractions (80). Certain beagle dogs displaying marked endogenous hyperlipoproteinemia and atherosclerosis even when fed low fat commercial diets were found to be hypothyroid. Administration of desiccated thyroid decreased serum cholesterol, triglycerides and lipid concentration affecting lipoprotein fractions at density  $<1.060$  (81). Thyroid hormones in man have also been reported to decrease plasma cholesterol while increasing cholesterol biosynthesis and catabolism (82).

#### Hypertension

of essential

In elucidating the etiology and pathogenesis of hypertension in man, various mechanisms or factors have been causally related to heightened blood pressure. Thus, the influence of neurogenic and psychologic factors have been widely recognized (83-85) as well as various hormonal factors (86-97). In man, hypertension has been variously related to primary aldosteronism (86-88), adrenal adenomas and hyperplasia (84,89), Cushing's Syndrome (83,90), etc., all resulting in increased adrenocortical secretions. Pheochromocytoma or tumors of the adrenal medulla wherein large amounts of epinephrine and norepinephrine are secreted (91) and norepinephrine have similarly been implicated in the pathogenesis of human and experimental hypertension (92). Excessive secretions by the thyroids (Graves disease) have likewise been associated and found to induce hypertension in man (93). Conversely, it is of interest that antithyroid treatment in rats provided significant protection against full development of renal hypertension (94). The renin-angiotensin system has also been involved in hypertension and regulation of blood pressure (88,95-97).

1003544390

Spontaneously Hypertensive Rats

In 1963 (98), a strain of spontaneously hypertensive rats (SHR) was selectively bred which has been considered most appropriate for studies relating to essential hypertension. Extirpative procedures as well as exogenous hormones administration have been used to demonstrate the active role played by the pituitary-adrenal and pituitary-thyroidal axis in inducing and maintaining the hypertensive state in the SHR strain (99-101). Other investigations have demonstrated a similar contributory role of the adrenal-medulla and increased catecholamine output with the development of the spontaneously hypertensive state (102-104). Studies with the hypertensive strain have similarly indicated that the severity of hypertension can be modified and accentuated by gross increases in Na and salt intake (104). No apparent increased activity has been noted in the renin-angiotensin system of the spontaneously hypertensive strain by some investigators (105). Recent studies (106-108), however have reported hypothyroidal function rather than hyperthyroidism (99-101) in the spontaneously hypertensive rats.

The spontaneously hypertensive rats have been found to develop hypertensive states exceeding 150 mm. Hg, within 2 months to 15 weeks with an incidence of 100%. The degree of hypertension has been found to increase with age and to reach levels as high as 200 mm Hg and over (98-101,103,109). The absence of abnormalities in the kidneys except at the terminal stages of hypertension (109) accompanied by the spontaneous nature and the endocrine resemblances have thus caused the investigators to consider this strain an excellent model for studies of essential hypertension (98, 101,109).

Analyses of the pathological changes in the blood vessels, heart (cardiac hypertrophy, etc.), kidneys and brain of the spontaneously hypertensive rats (109) have paralleled changes found in the cardiovascular pathology caused by essential hypertension in man. Similarly, pathological and histological changes noted in the pituitary, adrenals and thyroids of the SHR strain (110) have also paralleled endocrine and tissue changes correlated with hypertension in man. To illustrate, hypertrophy and "hyaline changes" were noted in the pituitary of the hypertensive rats, as well as hypertrophy of the glomerular and fascicular zones of the adrenals, etc. Augmented cerebrovascular (111) and cardiovascular (112) pathologies have been demonstrated in spontaneously hypertensive rats give 1% saline for drinking water with and without a high fat-cholesterol diet.

Spontaneously Hypertensive Rats and Cholesterol Diets

Umehara et al, (113) observing effects of high-fat diets, reported elevated total blood cholesterol values in spontaneously hypertensive rats as well as degenerative lesions in the peripheral arteries and arteriosclerotic changes in the arteries of the brain, heart and kidneys. In contrast, Nagaoka et al (114) claimed that feeding a high cholesterol diet (2%) plus 0.15% propylthiouracil or a diet of 2% cholesterol to spontaneously hypertensive rats demonstrated that cholesterol was not involved in the progression of hypertension nor in the occurrence of thrombus formation. Feeding of the combined cholesterol and propylthiouracil diet however, consistently elevated plasma cholesterol levels above control diet values. Plasma cholesterol levels of animals fed cholesterol alone were lower than the cholesterol - PTU group, but higher than in the spontaneously hypertensive rats fed the control diet. Studies involving diets containing 1% Na Cl or 1% Na Cl plus high-fat and cholesterol levels were reported to significantly induce fibrinoid necrosis of arterioles and arteries and increase pathology in the kidneys, hearts and brains of spontaneously hypertensive rats (112) as well as produce a high incidence of cerebrovascular diseases, infarcts and hemorrhages in the SHR (111).

1003544391

The present investigators have published investigations with hallucinogens such as lysergic acid diethylamide (115-119) and mescaline (120-124) on the metabolism behavior and endocrine function of rats and mice.

Dr. Weltman has also engaged in studies related to the effects of auditory stress (125-127), vibration stress (128-130), isolation stress (131-135) as well as behavioral, metabolic and physiological differences in audiogenic-seizure susceptible vs. resistant rats (136-138) and the excitable homozygous-whirler vs. normal, heterozygous-whirler mutant mice (139-145). The various behavioral, biochemical and endocrine studies have indicated heightened metabolism rates, increased adrenocortical function and, in general, inhibited gonadal activity in the whirler mice. These may be symptomatic and correlated with physiological and neuronal changes responsible for the wild, circling, locomotor activity. Biochemical alterations have indicated significantly increased plasma corticosterone (142,143), adrenal corticosterone (142,143) and adrenal catecholamine levels (143) accompanied by significant alterations in carbohydrate metabolism (142,143,145) (i.e., lower plasma glucose and liver glycogen levels). Although total plasma protein levels were significantly reduced due to depressions in  $\alpha_1$ ,  $\alpha_2$ , beta and gamma globulins, albumin levels were significantly higher (142).

1003544392

Item #8. Brief statement of working hypothesis (continued):

Similarly, while many investigators associate tobacco smoking with hypertension and coronary heart disease, epidemiological studies (33) have indicated that the average blood pressure levels were somewhat lower in smokers than nonsmokers. On the other hand, smoking has been reported to cause larger rises in blood pressures of hypertensive subjects than in normal subjects (3).

Our findings involving effects of 6 and 29 weeks of oral nicotine administration (2.28 mg/kg/day, equivalent to two packs of cigarettes per day) revealed significantly decreased total cholesterol levels in male spontaneously hypertensive rats and showed indications of somewhat lower blood pressure levels in the nicotine-treated or untreated SHR. Nicotine has also been found to lower the blood pressure levels of renal hypertensive rats (28).

While the present investigation is not devised to demonstrate mechanisms affecting cholesterol metabolism in the SHR, the detailed time-sequential studies involving blood lipoprotein, cholesterol, triglycerides, phospholipid and FFA will yield a clearer understanding of the effects of nicotine on cholesterol and lipid metabolism in the spontaneously hypertensive rats. Similarly, the intensive study should demonstrate possible synergistic, antagonistic etc. interaction and relationships of nicotine and/or cholesterol intake with hypertension, cholesterol and lipid metabolism and the possible development of vascular, arteriosclerotic and atherosclerotic pathologies. Comparable studies with the genetically related normotensive strain will indicate a measure of the intermediate and prolonged effects which nicotine and cholesterol pose to initially normotensive individuals as well as possible diversities in the response patterns of the hypertensive and normotensive strains to nicotine and/or cholesterol intake. There are no indications that investigations have been undertaken to compare and clarify effects of simultaneous nicotine and cholesterol administration on blood pressure, lipid profile and vascular pathology in spontaneously hypertensive rats.

1003544393

Item #9. Details of experimental design and procedures (continued):

of nicotine. Records will be maintained of the volumes consumed during the 18 hour period and plain tap water during the remaining 6 hour period. The 2.28 mg/kg/day dose has been reported to have the psychic equivalence of the daily intake of "two packs of cigarettes per day" (48). Since 4 rats will be caged in a unit, Group A animals will receive an average rather than an exact daily dose which they would have received if they were injected. The oral dose and method, although not exact, is preferable since it simulates the gradual daily smoking of cigarettes; it avoids the stress of too frequent injections and the possibility of convulsive doses being administered via the injection procedures. The nicotine alkaloid will be obtained from Eastman-Kodak.

Group B animals will be supplied a cholesterol enriched diet mix consisting of 5% cholesterol and 95% Purina Laboratory Chow Meal. To insure even distribution of the cholesterol, the ingredients will first be mixed in a mortar followed by agitation in a container on a horizontal shaker until uniformly dispersed (20 min.).

Group C animals will receive equivalent oral doses of nicotine and the cholesterol-diet mix furnished to Group B. Group D will represent the untreated control spontaneously hypertensive group. In all cases, the procedure for changing bottles containing nicotine and/or water alone will be followed in all groups to make sure that all cages and groups receive identical handling or disturbances.

The following schematic outline will represent the population sizes for the Series I (spontaneously hypertensive rats) and Series II (normotensive rats) studies:

Series I - - SHR

Group 1 - - Six months

Group A - nicotine group (dose 2.28 mg/kg/day) - 16 rats

Group B - cholesterol enriched diet mix (5%) - 16 rats

Group C - nicotine (2.28 mg/kg/day); cholesterol diet (5%) - 16 rats

Group D - control - tap water - 16 rats

Group 2 - - One 1/2 years

Group A - nicotine - 20 rats

Group B - cholesterol diet - 20 rats

Group C - nicotine-cholesterol diet - 20 rats

Group D - control

Group 1 will contain a total of 64 rats and Group 2 of Series I a total of 80 rats consisting of 144 rats. The longer chronic study will consist of a larger population in view of the possibility of premature deaths.

Group 1 (6 months) and Group 2 (1 1/2 years) of the Series II study (normotensive rats, WKY/N) will have the same schematic outline and population sizes. Thus, a total population of approximately 288 animals will be used in the present proposal.

During the prolonged 1 1/2 year chronic investigations, heparinized blood samples will be collected from the orbital sinus (147) at the end of 2, 6, 12, 18, 24, 32, 40, 52 and 64 weeks of treatment. Plasma samples will be assayed for total cholesterol (148), triglycerides (149), phospholipids (150), glucose (151) and total plasma protein (152) titers. Lipoprotein assays (153) will be determined by use of a Helena Laboratories electrophoretic equipment and densitometer (Electrophoresis Chamber and Quick Scan Densitometer, Model 1111). The preceding analyses will be determined on an individual basis since small plasma volumes are required for the respective analyses. FFA (154) determinations will be derived by pooling the remaining blood of 3 plasma samples for one FFA assay due to the larger volume of plasma required for the FFA quantitative procedures.

At appropriate intervals, heparinized plasma samples will be assayed for nicotine and metabolite titers (i.e., cotinine etc.) using a Perkins Elmer Model 811 gas chromatograph equipped with an hydrogen flame ionization detector and a Speedomax G recorder (155). At time of sacrifice, liver specimens will likewise be assayed for nicotine and metabolite levels. These tests will assist in estimating and correlating the level of nicotine intake

1003544394

with the effects of nicotine on the various parameters in the nicotine and nicotine-cholesterol-treated rats.

To determine the effects on endocrine organs, as well as possible pathological alterations due to the nicotine and/or cholesterol regimen, aliquot and representative populations of the spontaneously hypertensive and normotensive group will be sacrificed after 24 weeks of treatment. Heparinized plasma samples will be collected following rapid decapitation with a Harvard decapitator. Similarly, at the end of the 1½ year testing period (78 weeks), the remaining groups will be similarly decapitated for plasma collections, organ weight as well as gross and subsequent histological evaluations of pathological alterations.

Systolic blood pressure measurements will be obtained from the respective tests and control groups prior to initiation of the agents (0 weeks) and following 4,8,16, 23,30,38,50,62 and 77 weeks of treatment. Animals to be sacrificed will have their blood pressures determined 1 week prior to sacrifice to reduce the possibilities of stress effects on biochemical parameters.

Upon sacrifice at the 24 and 78 week periods, the animals will be rapidly dissected for observation of effects of nicotine and/or cholesterol on adrenals, thymus, spleen, liver, testes, seminal vesicles, kidneys, heart and brain weights. Decreases or involution of the thymus and spleen lymphoid organs have been used as a measure of increased adrenocortical and steroidal output. Alterations in the testes and seminal vesicle weights will reflect gonadal influences by the respective treatment. The body cavities, brain, lungs, kidneys, heart and vascular system will be examined for gross pathological abnormalities resulting from hypertensive, arteriosclerotic and atherosclerotic alterations. Tissues and organs such as the heart, kidneys, pulmonary artery, aorta, renal blood vessels, brain, lungs etc. will be fixed in 10% formalin solution for histological and microscopic evaluations of arteriosclerotic and atherosclerotic pathological anomalies. Among other factors animals will be examined for cardiac infarctions (scarring and hypertrophy) periarteritis nodosa, nephrosclerosis, cerebral hemorrhage, lung involvements, etc.

\* Note:

The present proposal does not include in the budget the costs for histological processing of the tissues and organs. As indicated, at the conclusion of the 6 month and subsequent 1½ year studies, the tissues will be fixed in 10% formalin. If biochemical, blood pressure, organ weight and autopsy data then indicate the merit for further histological examination, this phase will be pursued subject to the consent of the Council for Tobacco Research and a supplemental budget request to be submitted for this histological work.

In all series and groups, records be maintained of premature deaths. Whenever possible animals will be autopsied and examined grossly for pathological abnormalities and probable cause of death. Tissues and organs will be selected for histological examination when periods between time of death and final autopsy are not prolonged.

The various data will be analyzed by standard t-test procedures and analyses of variance (156) to determine significant effects of nicotine and/or cholesterol treatments in the spontaneously hypertensive and normotensive groups. Thus, body weight, food consumption, water consumption, food utilization ratios, blood pressure, organ weights and the various biochemical parameters will be compared by these procedures. Correlation procedures (156) will also be used to analyze i.e. cholesterol and blood pressure levels etc. and to determine direct or inverse relationships of the various biochemical parameters due to nicotine and/or cholesterol administration.

The laboratory has available a Cogito 566 P R model calculator(Marchant) as

1003544395

well as Friden 130 Electronic Calculators for computation of the data. In addition, the facilities of the Long Island University Brooklyn Center, Computer Center are available. The Computer Center has an IBM Model #1130-3 C computer and accessories for statistical analyses involving variance, correlations etc. The services of Mr. Anthony Brogna, Director of the Computer Center and the equipment are consequently available to us.

1003544396

REFERENCES

1. Karvonen, M., Orma, E., Keys, A., Fidanza, F. and Brozek, J.: Lancet 1:492, 1959.
2. Damon, A.: Science 134:339, 1961.
3. Roth, G. M. and Schick, R. M.: Ann. N. Y. Acad. Sci. 90:308, 1960.
4. Blackburn, H., Brozek, J., Taylor, H. L. and Keys, A.: Ann. N. Y. Acad. Sciences 90:277, 1960.
5. Hines, E. A.: Ann. N. Y. Acad. Sciences 90:333, 1960.
6. Thomas, C. B.: Ann. Internal Med. 53:697, 1960.
7. Thomas, C. B. and Murphy, E. A.: Ann. N. Y. Acad. Sci. 90:266., 1960.
8. von Ahn, B.: Ann. N. Y. Acad. Sciences 90:190, 1960.
9. Kershbaun, A., Billet, S. and Khorsandian, R.: Amer. Heart Jour. 69:206, 1965.
10. Kerrigan, R., Jain, A. C. and Doyle, J. T.: Amer. Jour. Med Sciences 255:113, 1968.
11. Kershbaun, A. and Billet, S.: Geriatrics 21:155, 1966.
12. Auerbach, O., Hammond, E. C. and Garfinkel, L.: New Eng. Jour. Med. 273, 775, 1965.
13. Hammond, E. C.: Jour. Nat. Cancer Instit. 32:1161, 1964.
14. Hadley, H. G.: Med. Rec. 153:267, 1941.
15. Hammond, E. C. and Horn, D.: Jour. Amer. Med. Assoc. 166:1294, 1958.
16. Fredrickson, D. S. and Levy, R. I.: (In Stanbury, J. B., Wyngaarden, J. B. and Fredrickson, D. S., editors): Metabolic basis of inherited disease, 3rd ed., New York, 1972, McGraw-Hill, pp. 531-614.
17. Kayden, H. J.: Amer. Heart Jour. 85:422, 1973.
18. Keys, A., Taylor, M. L., Blackburn, H., Brozer, T., Anderson, J. R. and Simonson, E.: Circulation 28:381, 1963.
19. Heinle, R. A., Levy, R. I., Fredrickson, D. S., and Gordon, R.: Amer. Jour. Cardiol. 24:178, 1969.
20. Greenhalgh, R. M., Lewis, B., Rosengarter, D. S., Clannan, J. S., Mervart, I. and Martin, P.: Lancet 2:947, 1971.
21. Farid, N. R.: Amer. Heart Jour. 85:430, 1973.
22. Farid, N. R. and Anderson, J.: Lancet 1, 1398, 1972.
23. Fredrickson, D. S. and Lees, R. S.: Circulation 31:321, 1965.

1003544397

24. Fredrickson, D. S. and Lees, R. S.: "Familial Hyperlipoproteinemia" in "The Metabolic Basis of Inherited Disease", McGraw-Hill, New York, 1966, pp. 429-485.
25. Nerenburg, S. T.: "Hyperlipoproteinemias", University of Illinois Medical Center Monograph, Aug. 25, 1970, pp. 1-14.
26. Levy, R. I. and Fredrickson, D. S.: Amer. Jour. Cardiology 22:576, 1968.
27. Wenzel, D. G., Wattanapongsiri, A. and Vedral, D.: Jour. Pharm. Exp. Therap. 145:315, 1964.
28. Wenzel, D. G. and Azmeh, N.: Arch. Internat. Phar. Therap. 187:367, 1970.
29. Weltman, A.S., Pandhi, V., Kraus, S. D. and Johnson, L.: Fed. Proc. 32:806, 1973.
30. Weltman, A.S., Kraus, S.D., Pandhi, V., Johnson, L. and Vaidya, R.: Fed. Proc. 33:359, 1974.
31. Weltman, A.S., Kraus, S.D. and Pandhi, V.: "Acute effects of nicotine in spontaneously hypertensive rats". To be presented Amer. Pharm. Assoc. Acad. of Pharm. Sci. Meeting (121st) Chicago, Aug. 3-9, 1974.
32. Brozek, J. and Keys, A.: Science 129:1203, 1957.
33. Seltzer, C.C.: Amer. Heart Jour. 87:558, 1974.
34. Simon, D. L. and Iglauder, A.: Ann. N. Y. Acad. Sci. 90: 119, 1960.
35. Armitage, A.K.: Brit. Jour. Pharmacol. 25:515, 1965.
36. Haag, H. B., Larson, P. S. and Weatherby, J. H.: Ann. N. Y. Acad. Sci. 90:227, 1960.
37. Wenzel, D. G., Kamal, J. S. and Turner, J. A.: Ann. N. Y. Acad. Sci. 90:302, 1960.
38. Bhagat, B.: Brit. Jour. Pharmacol. 38:86, 1970.
39. Westfall, T. C.: European Jour. Pharmacol 10:19, 1970.
40. Kin, H.: Jap. Jour. Med. Sci. Pharm. 12:66, 1940.
41. Thienes, C. H.: Ann. N. Y. Acad. Sci. 90:239, 1960.
42. Puri, P. S., Alamy, D. and Bing, R. J.: Jour. Clin. Pharmacol. 295:295, 1968.
43. Watts, D. T. : Ann. N. Y. Acad. Sci. 90:74, 1960.
44. Comroe, J. H.: Ann. N. Y. Acad. Sci. 90:48, 1960.
45. Larson, R. K., Fukuda, P. and Murray, J. F.: Amer. Rev. Respir. Dis. 91:556, 1965.
46. Gofman, J. W., Lindgren, F. T., Strisower, B., de Lalla, O. Glazier, F., Tamplin, A.: Geriatrics 10:349, 1955.

1003544398

47. Murchison, L. E. and Fyfe, T.: Lancet 2, 182, 1966.
48. Wenzel, D. G. and Beckloff, G. L.: Jour. Amer. Pharm. Assn. 47:338, 1958.
49. Maslova, K. K.: Bull. Exper. Biol. & Med. 41:20, 1956.
50. Kershbaum, A.: Acta Cardiol. 23:317, 1968.
51. Havel, R. J. and Goldfien, A.: Jour. Lipid Res. 1:102, 1959.
52. Barrett, A. M.: Brit. Jour. Pharmacol. 22:577, 1964.
53. Kirschbaum, A., Billet, S., Hirabayashi, M. and Feinberg, L. J.: Jour. Amer. Med. Assn. 201:545, 1967.
54. Kershbaum, A., Pappajohn, D. J., Billet, S., Hirabayashi, M. and Shafiiha, H.: Jour. Amer. Med. Assn. 203:275, 1968.
55. Czochra-Lysanowicz, A., Gorski, M. and Kedra, M.: Ann. Univ. Maria Curie-Sklodowska (Med) 14:181, 1959.
56. Stefanovich, V., Gore, I., Kajiyama, G. and Iwanaga, Y.: Exper. Molecular Path. 11:71, 1969.
57. Wenzel, D. G., Turner, J. A., Jordon, S. W. and Singh, J.: Circulation Res. 9:694, 1961.
58. Svacha, A. J., Wesson, N. C. and Waslien, C. I.: Fed. Proc. 33:690, 1974.
59. Nakazawa, R.: Japan. Jour. Med. Sci. Pharmacol. 5:109, 1931.
60. Wilson, R. H. and De Eds, F.: Jour. Ind. Hyg. Toxicol. 18:553, 1936.
61. Pechstein, L. A. and Reynolds, W. R.: Jour. Comp. Psychol. 24:459, 1937.
62. Haag, H. B., Weatherby, J. H., Fordham, D. and Larson, P. S.: Fed. Proc. 5:181, 1946.
63. Stadlander, K. H.: Z. ges. exptl. Med. 99:670, 1936.
64. Kin, S. S.: Japan. Jour. Med. Sci. Pharmacol. 10:59, 1937.
65. Kobayaski, S.: Chem. Abstr. 31:6734, 1937.
66. Essenberg, J. M., Schwind, J. V. and Patras, A. K.: Jour. Lab. Clin. Med. 25:708, 1940.
67. Willson, J. R.: Amer. J. Obs. Gyn. 43:839, 1942.
68. Wilson, R. H., McNaught, J. B. and De Eds, F.: Jour. Ind. Hyg. 20:468, 1938.
69. Anitschkow, N.: Pathologische Anatomie und allgemeine Pathologie der Arteriosklerose. Compt. rend. 2<sup>e</sup>, Conf. Internat. Path. Geograph, Utrecht, July 1934, pp. 44-97.
70. Modrak, J. B. and Langner, R.: Fed. Proc. 33:230, 1974.
71. Dauber, D. V. and Katz, L. N.: Arch. Path. 34:937, 1942.

1003544399

72. Dauber, D. V. and Katz, L. N.: Arch. Path. 36:473, 1943.
73. Horlick, L. and Katz, L. N.: Amer. Heart Jour. 38:336, 1949.
74. Wong, H. Y. C., David, S. N., Orimilikwe, S. O., Udoh, N. C. and Johnson, F. B.: Fed. Proc. 33:677, 1974.
75. Wilson, R. B. and Hartroft, W. S.: Jour. Atherosclerosis Res. 8:945, 1968.
76. Wilson, R. B. and Hartroft, W. S.: Arch. Path. 89:457, 1970.
77. Wilson, R. B., Hartroft, W. S., Conen, P. E. and Newberne, P. M.: Arch. Path. 91:307, 1971.
78. Wexler, B. C.: Jour. Atheroscler. Res. 4:57, 1964.
79. Wexler, B. C. and Kittinger, G. W.: Jour. Path. and Bact. 94:231, 1967.
80. Kuehl, K., Roheim, P. S. and Eder, H. A.: Fed. Proc. 33:351, 1974.
81. Manning, P. J. and Middleton, C. C.: Fed. Proc. 33:235, 1974.
82. Heftmann, E. and Mossettig, E.: Biochemistry of Steroid, Reinhold Pub., N. Y. 1960, p 11.
83. Abe, T.: Jap. Circulation Jour. 30:1387, 1966.
84. Mallin, S. R.: Ann. Intern. Med. 70:69, 1969.
85. Thomas, C. B.: Current Med. Digest 36:472, 1969.
86. Conn, J. W.: Jour. Lab. Clin. Med. 45:3, 1955.
87. Conn, J. W.: Arch. Int. Med. 107:813, 1961.
88. Newton, M. A. and Laragh, J. H.: Endocrin. 28:1014, 1968.
89. Kokko, J. P.: Lancet 1:468, 1967.
90. Plotz, C.M., Knowlton, A. I. and Ragan, C.: Amer. Jour. Med. 13:597, 1952.
91. Turner, C. D.: Gen. Endocrinology, W. B. Saunders, Philadelphia 1960, p 444.
92. Mendlowitz, M., Geltow, S. E., Wolf, R. L. and Tochman, J.: Amer. Heart Jour. 70:677, 1965.
93. Williams, R. H. and Bakke, J. L.: Textbook of Endocrinology, W. B. Saunders, Philadelphia, 1962, pp 150-196.
94. Waters, I. W., Fregly, M. J. and Voss, E.: Tox. Applied Pharmacol. 10:165, 1967.
95. Mulrow, P. J., Bartter, F. C., Kirkendall, W. M., Peterson, R. E. and Tait, J. F.: Circulation 40:739, 1969.
96. Krakoff, L. R., Goodwin, F. J., Baer, L., Torres, M. and Laragh, J. H.: Circulation 42:335, 1970.
97. Romero, J. C. and Hoobler, S. W.: Amer. Heart Jour. 80:701, 1970.

1003544400

98. Okamoto, K. and Aoki, K.: Jap. Circulation Jour. 27:282, 1963.
99. Aoki, K.: Jap. Heart Jour. 4:443, 1963.
100. Aoki, K.: Jap. Heart Jour. 4:561, 1963.
101. Aoki, K.: Jap. Heart Jour. 5:57, 1964.
102. Morisawa, T.: Jap. Circulation Jour. 32:161, 1968.
103. Ozaki, M., Suzuki, Y., Yamori, Y. and Okamoto, K.: Jap. Circulat. Jour. 32:1367, 1968.
104. Louis, W. J., Tabei, R., Spector, S. and Sjoerdsma, A.: Circulation Res. 24:93, 1969, Supplement I.
105. S. Koletsky, P. Shook and Rivera-Velez, J.: In "Spontaneous Hypertension, its Pathogenesis and Complications" ed. K. Okamoto, Springer-Verlag, New York, 1972, p. 199.
106. Manger, W. M. and Werner, S. C.: Fed. Proc. 32:749, 1973.
107. Manger, W. M., Werner, S. C., Freedman, L. S., Dufton, S. and von Estorff: Fed. Proc. 33: 543, 1974.
108. Fregly, M. J.: Fed. Proc. 33:359, 1974.
109. Okamoto, K., Aoki, K., Nosaka, S. and Fukushima, M.: Jap. Circulat. Jour. 28: 943, 1964.
110. Aoki, K., Tankawa, H., Fujinami, T., Miyazaki, A. and Hashimoto, Y.: Jap. Heart Jour. 5:426, 1963.
111. Okamoto, K., Hazama, F., Haebara, H., Amano, S., Tanaka, T. and Ooshima, A.: In "Spontaneous Hypertension, its Pathogenesis and Complications", ed. K. Okamoto, Springer-Verlag, New York, 1972, p 129.
112. Hazama, F., Tanaka, T., Ooshima, A., Haebara, H., Amano, S., Yamazaki, Y. and Okamoto, K.: In "Spontaneous Hypertension, its Pathogenesis and Complications", ed. K. Okamoto, Springer-Verlag, New York, 1972, p. 134.
113. Umehara, Y., Sasaki, A., Kudo, Y. and Mori, T.: In "Spontaneous Hypertension, its Pathogenesis and Complications", ed. K. Okamoto, Springer-Verlag, New York, 1972, p. 142.
114. Nagaoka, A., Kikuchi, K., Kawaje, H., Matsuo, T. and Aramaki, Y.: In "Spontaneous Hypertension, its Pathogenesis and Complications", ed. K. Okamoto, Springer-Verlag, New York, 1972, p. 149.
115. Sackler, A. M., Weltman, A. S. and Owens, H.: Nature 198:1119, 1963.
116. Sackler, A. M., Weltman, A. S. and Sparber, S. B.: Nature 199:1194, 1963.
117. Weltman, A. S. and Sackler, A. M.: J. Pharm. Sci. 54:1382, 1965.
118. Weltman, A. S. and Sackler, A. M.: J. Endocrinol. 34:81, 1966.
119. Sackler, A. M., Weltman, A. S. and Owens, H.: Toxicol. Applied Pharmacol. 9:324, 1966.

1003544401

120. Weltman, A. S., Sackler, A. M. and Schwartz, R.: Amer. Zool. 8:753, 1968.
121. Weltman, A. S., Sackler, A. M. and Schwartz, R.: Exp. Med. Surg. 26:187, 1968.
122. Weltman, A. S., Sackler, A. M. and Schwartz, R., Johnson, L. and Steinglass, P.: Amer. Zool. 9:1079, 1969
123. Weltman, A. S., Sackler, A. M. and Johnson, L.: J. Pharm. Sci. 59:1659, 1970.
124. Sackler, A. M. Weltman, A. S. and Johnson, L.: Exp. Med. Surg. 29:118, 1971.
125. Jurtshuk, P., Jr., Weltman, A. S., and Sackler, A. M.: Science 129:1425, 1959.
126. Sackler, A. M., Weltman, A. S., Bradshaw, M. and Jurtshuk, P., Jr.: Acta Endocrinologica 31:405, 1959.
127. Sackler, A. M., Weltman, A. S. and Jurtshuk, P., Jr.: Aerospace Med. 31:749, 1960.
128. Weltman, A. S., Sackler, A. M., Owens, H. and Bernstein, M.: Amer. Zoologist 3:526, 1963.
129. Sackler, A. M. and Weltman, A. S.: Aerospace Med. 37:158, 1966.
130. Weltman, A. S., Sackler, A. M., Gennis, J. and Steinglass, P.: The Physiologist 9:318, 1966.
131. Weltman, A. S., Sackler, A. M., Sparber, S. B. and Opert, S.: Fed. Proc. 21: 184, 1962.
132. Weltman, A. S., Sackler, A. M. and Sparber, S. B.: Aerospace Med. 37:804, 1966.
133. Weltman, A. S., Sackler, A. M., Schwartz, R. and Owens, H.: Laboratory Animal Care 18:426, 1968.
134. Sackler, A. M., Weltman, A. S., Schwartz, R. and Steinglass, P.: Acta Endocrinologica 62:367, 1969.
135. Weltman, A. S., Sackler, A. M. and Schwartz, R.: Life Sciences 9:291, 1970.
136. Sackler, A. M., Weltman, A. S., Owens, H., Kreger, A. S. and Jacobs, R.: Amer. Zool. 2:553, 1962.
137. Sackler, A. M., Weltman, A. S. and Kreger, A. S.: Exp. Med. Surgery 24:258, 1966.
138. Weltman, A. S., Sackler, A. M. and Owens, H.: Physiology and Behavior 3:281, 1968.
139. Sackler, A. M., Weltman, A. S., Steinglass, P., and Kraus, S. D.: Fed. Proc. 23:252, 1964.
140. Weltman, A. S. and Sackler, A. M.: Proc. Soc. Exp. Biol. Med. 123:58, 1966.
141. Sackler, A. M. and Weltman, A. S.: Jour. Exp. Zool. 164:133, 1967.
142. Weltman, A. S., Sackler, A. M., Johnson, L., and O'Conner, G.: Fed. Proc. 29:778, 1970.
143. Weltman, A. S., Sackler, A. M., Lewis, A. S. and Johnson, L.: Physiol. & Behavior 5:17, 1970.

1003544A02

144. Weltman, A. S. and Sackler, A. M: Acta Endocrinologica 64:347, 1970.
145. Sackler, A. M. Weltman, A. S.: Experientia 26:369, 1970.
146. DeChamplain, J., Krakoff, L. R. and Axelrod, J.: Circulation Res. 20:136, 1967.
147. Grice, H. C.: Lab. Animal Care 14:483, 1964.
148. Zak, B., Dickerman, R. C., White, E., Burnett, H. and Cherney, P. J.: Amer. Jour. Clin. Path. 24:1307, 1954.
149. Phillips, R. E.: The Fluormetric determination of serum triglycerides. Manual of G. K. Turner Assoc. Inc.
150. Zilversmit, D. B. and Davis, A. K.: Jour. Lab. Clin. Med. 35:155, 1950.
151. Saifer, A. and Gerstenfeld, S.: Jour. Lab. Clin. Med. 51:448, 1958.
152. Sunderman, F. W.: Jour. Biol. Chem. 153:139, 1944.
153. Blood lipoprotein procedure outlined by Helen Inc. for use with their instrumentation and solutions.
154. Goss, J. E. and Lein, A.: Clin. Chem. 13:36, 1967.
155. Cymerman-Craig, J., Mary, N. Y., Goldman, N. L. and Wolf, L.: Jour. Amer. Chem. Soc. 86:3866, 1964.
156. Snedecor, G. W.: Statistical Methods, Iowa State College Press. Ames, 1949.

1003544403

Item #10. Space and facilities available (continued):

assays, a Perkins Elmer Model 811 Gas Chromatograph with a hydrogen flame ionization detector and a Speedomax G recorder is available for nicotine and nicotine metabolite assays. Helena Laboratories Inc. Electrophoretic and Densitometer equipment (Electrophoretic Chamber and Quick Scan Densitometer, Model 1111) are available for the lipo-protein assays.

The animals are housed in an air-conditioned room approximately 22' x 27' (594 sq. ft.), provided with an exhaust system, which can contain 8-9 animal racks. Cages for mice or rats are available depending upon the particular study. The animal room contains water facilities and a drainage system for proper sanitary needs. Adjacent to the animal room are 2 storage rooms approximately 5' x 10' (50 sq. ft.) and 5' x 13' (65 sq. ft.). These are used to store food, shavings and other sundry supplies. Also adjacent to the animal quarters is a behavioral study room 7' x 12' (84 sq. ft.) used for O<sub>2</sub> consumption, locomotor activity and blood pressure studies. This room permits animals to be observed and studied in relative quiet. A separate room 10' x 17' (170 sq. ft.) removed from the animal room by a corridor and 2 doors serves as office space and area for auditory stress studies. This separation prevents extraneous noise from bells, etc., to reach and disturb animals in the animal quarters. A washroom, approximately 12' x 17' (204 sq. ft.) contains an automatic-spray washing machine and sinks which are used to sterilize and cleanse cages and water bottles. The main research laboratory approximately 16' x 50' (800 sq. ft.) is provided with desks, table tops, cabinets and much of the equipment cited above. This room contains 3 water-sinks and is the area where autopsies, hematological, histological, and biochemical tests are performed and where calculations are done.

- b) Institute of Pathology  
Downstate Medical Center, S. U. N. Y.  
450 Clarkson Avenue  
Brooklyn, N. Y.

At the Institute of Pathology, laboratory rooms and equipment are available for sectioning and automatic fixing and staining of the preparations and slides. They consist of microtomes, auto-technicons, microscopic equipment, glassware and accessory supplies. An electron microscope and fluorescent apparatus are available if these techniques are needed.

1003544A04